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EFFECT OF BEHAVIORAL AND PHYSIOLOGICAL VARIABLES ON MELATONIN SECRETION IN HUMANS

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ABSTRACT

The temporal pattern of melatonin secretion was studied in two populations of normal healthy adult volunteers in the Clinical Research Center (CRC) at M.I.T. In the first study, 15 male subjects were admited individually to the CRC for 36 hours on three different occasions, at least two weeks apart. On each occasion, the subject was exposed during the day to one of three different lighting conditions: bright full spectrun light dim full spectrum light or dim red light. Blood samples were then collected to monitor the temporal pattern of the subsequent nocturnal secretion of melatonin. In the second study, 5 normal female subjects of childbearing age were each admitted to the CRC on 3 different occassions and blood samples were collected over 24-hour periods at the begining, middle and end of the menstrual cycle. In both studies, there was very marked inter-individual differences in the amplitude, phase, and total amounts of melatonin secreted. However, in both studies, each individual repeated his characteristic melatonin pattern with remarkable fidelity. we will discuss these results in view of other recent studies of human and experimental animal melatonin patterns; compare and contrast typical approaches to the study of melatonin rhythms in animal and human subjects; and suggest an approach to the study of human melatonin.

KEY WORDS

Human pineal gland; light; circadian rhythm; diurnal animals; nocturnal animals

INTRODUCTION

Because the phasing and duration of nocturnal melatonin secretion are of demonstrable physiological and behavioral significance in certain experimental animals (Reiter, 1983; Tamarkin, et al., 1985; Goldman, 1983) it is not unreasonable to assume that these parameters may have physiological and behavioral significance in man. If so, the factors that determine the magnitude and time course of nocturnal melatonin secretion must be established.

Alternating periods of light and darkness were implicated in the regulation of pineal function even before the pineal hormone had been identified. It was shown that environmental illumination exerts an influence on volumetric and cytologic

aspects of the mammalian pineal (Quay, 1956). The earliest studies of the relationship between environmental illumination and pineal function involved work with intact and surgically blinded animals, or animals that were maintained for protracted periods of time either in continuous total darkness or in continuous laboratory illumination. Such studies clearly established that the rate of melatonin synthesis in the pineal gland is influenced by the photic environment (Wurtman, et al., 1963; 1964). They also demonstrated that the cyclic synthesis and secretion of melatonin is an inherently rhythmic phenomenon, driven by an endogenous circadian oscillator, which, in turn, is paced (entrained) by daily alternating periods of light and darkness (Ralph, et al., 1971; Reiter, et al., 1971). In this respect, rhythmic pineal function resembles numerous other biological variables that are similarly influenced by, and harmoniously coordinated with geophysically determined daily and seasonal changes in the environment (Wolf, 1962).

The precise nature, location, and control of the mammalian pineal's pacemaker is presently the focus of intense research. The involvement of the lateral eyes (Wurtman, et al., 1963), the retinohypothalamic optic tract (Moore and Lenn, 1972), and the suprachiasmatic nuclei of the hypothalamus (Moore, 1978) are clearly established. It is also clear that the daily pattern of environmental illumination, acting through these anatomical components, entrains (temporally coordinates) rhythmic melatonin secretion such that melatonin production and secretion is increased during the night and diminished during the day. In addition, the experimental exposure of animals to a light pulse during the daily dark period results in acute suppression of melatonin synthesis and secretion (Illnerova and Vanecek 1979) and a phase shift in the ensuing rhythmic pattern of secretion (Illnerova and Vanecek, 1981). The direction and magnitude of the phase shift is determined by timing of the light pulse.

Recent advances in bioanalytical methodology, particularly the development of highly sensitive and specific radioimmunoassay methods for the measurement of melatonin in body fluids have made possible the study of pineal function in human subjects (Lynch, 1980). Numerous laboratories around the world are now measuring melatonin in human blood, urine, cerebrospinal fluid, and saliva. It has been shown that the human pineal gland, like that of other animals, rhythmically synthesizes and secretes melatonin; that melatonin secretion is increased at night; and that nocturnal melatonin can be suppressed by exposing people to light of sufficient intensity (Lewy, et al., 1980). A curious phenomenon, commonly observed in studies of human melatonin secretion, is a very marked inter-individual variability in the amplitude, phase, and total amounts of melatonin observed in different individuals. Here we report some of our own experience with this phenomenon; speculate on the probable cause; and suggest an experimental approach that might mitigate the problem.

METHODS AND MATERIALS

Subjects

As part of more extensive studies on the relationship between certain behavioral and physiological variables and concomitant changes in endogenous melatonin levels that will be reported in detail elsewhere, we have monitored 24-hour melatonin profiles in two populations of normal healthy adult volunteers. In the first study, 15 adult male subjects (aged 18-40 years) were admitted individually to the Clinical Research Center (CRC) for two nights and one day on three different occasions, at least two weeks apart. The first night each subject slept in darkness from 11:30 pm until 7:30 am. He was then exposed during the day to one of three different lighting conditions: bright full spectrum light (2500 lux), dim

full spectrum light (100 lux), or dim red light (150 lux). Blood samples were then collected at 2-hour intervals from 4 pm throughout the ensuing 8-hour period of sleep and darkness. In the second study, 5 normal female subjects (aged 19-28 years) with regular menstrual periods were admitted to the CRC for 24 hours on three separate occasions. Blood samples were collected at 2-hour intervals throughout the day and night during the follicular, periovulatory, and luteal phase of each subject's menstrual cycle. Blood samples were allowed to clot in the refrigerator, serum was separated by centrifugation and stored frozen until assayed for melatonin concentration.

Melatonin assay

Melatonin concentrations were measured in duplicate 1 ml samples of serum by radioimmunoassay (RIA) using CIDtech Ultraspecific Melatonin Antiserum (CIDtech Research Inc., Hamilton, Ontario; Brown, et al., 1983). One ml serum samples were extracted into 5 ml of chloroform; the chloroform extract was then evaporated to dryness under a stream of nitrogen and the residue was redissolved in 0.5 ml of phosphate buffer (pH 7.5) containing 0.1% gelatin, and then washed with 1 ml of petroleum ether. The buffered extracts of serum samples and buffer samples containing graded concentrations of authentic melatonin were then combined with 100 ul of antiserum solution (diluted to 1:6000) and 100 ul (3000 cpm) of ^{3}H-melatonin (New England Nuclear Co., Boston MA). After incubating for one hour at 37°C, 0.7 ml of saturated ammonium sulfate was added; the mixture was then incubated overnight at 4°C, and antibody-bound ^{3}H-melatonin was collected as a precipitate by centrifugation. Radioactivity was then measured in a liquid scintillation counter, and melatonin concentrations were estimated by means of the logit-log plot (Rodbard, 1974). When 50 or 100 pg/ml of authentic melatonin was added to samples of pooled serum, recoveries in the range of 96 - 100% were obtained. The intraassay coefficients of variation were 8.1% and 10% respectively. The corresponding interassay coefficients of variation were 17.3% and 7.3%. The sensitivity of the assay (defined as twice the standard deviation of maximum binding) was 5 pg/ml (22 pmol/l).

RESULTS

Normal male subjects

The temporal pattern of the nocturnal increase in circulating melatonin levels was measured in each of 15 male subjects following daytime exposure to one of three different lighting conditions. Figure 1 shows the mean nocturnal melatonin pattern of all 15 subjects following exposure to bright full spectrum light (2500 lux) and dim ful spectrum light (100 lux). The pattern produced with exposure to dim red light was indistinguishable from the other two and was omitted from the figure in the interest of simplicity. Clearly, daytime exposure to different lighting conditions within this intensity range did not significantly affect the amplitude, time course, or total amount of the ensuing nocturnal melatonin secretion. The nocturnal melatonin profiles of four of the subjects, chosen to illustrate the individual diversity within this group, are shown separately in Figure 2. Here the mean $\pm$ S.E. of serum melatonin concentrations measured at corresponding 2-hour time points following each of the three different lighting conditions is shown. The small standard errors observed at each time point demonstrate the remarkable precision with which each subject replicated his characteristic melatonin secretory pattern in spite of the changes in daytime light intensity. There is, however, very considerable variation among the individuals in peak melatonin levels, time courses, and total amounts of melatonin secreted during the 16-hour period.

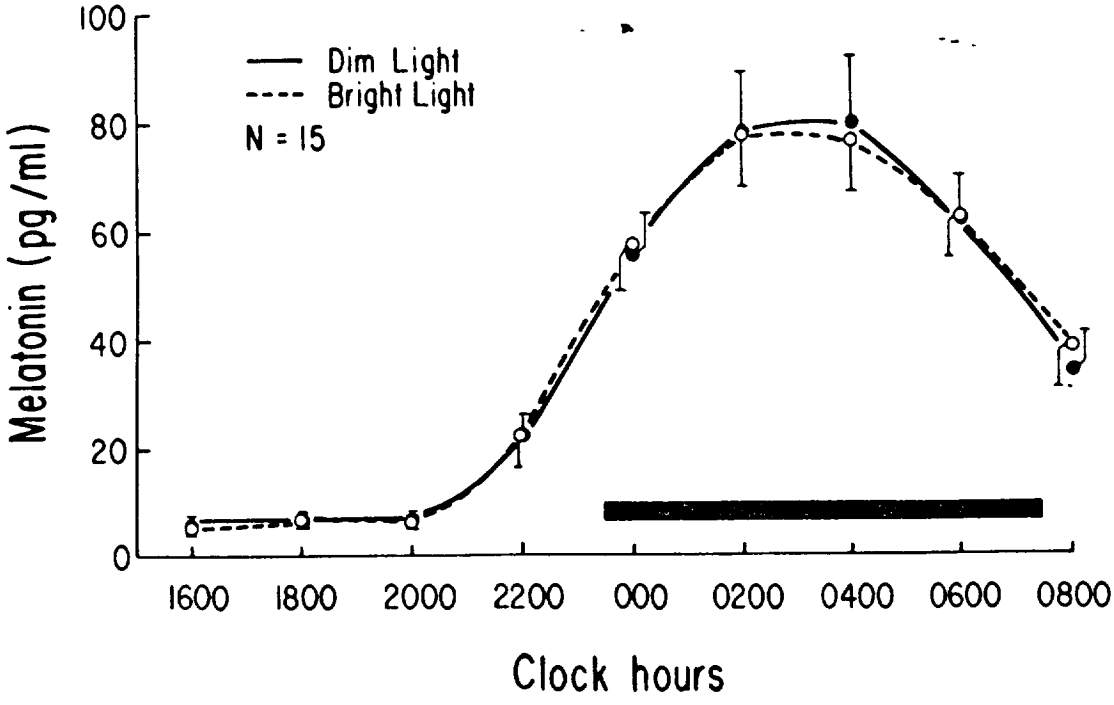

Fig. 1 Serum melatonin levels in 15 male volunteers sampled at 2-hour intervals from 4 pm to 8 am. Subjects were exposed either to bright ---0--- or dim —●— full spectrum light (2500 or 100 lux) from 7:30 am, then slept in darkness between 11:30 pm and 7:30 am the following morning. Vertical bars indicate one standard error of the mean. The horizontal bar indicates the period of sleep and darkness.

Normal female subjects

The 24-hour profile of melatonin secretion was assessed in 5 normally cycling women during the follicular, periovulatory, and luteal phases of each subject's menstrual cycle. The mean daily melatonin secretory pattern (Fig. 3) is in agreement with the generally accepted features of melatonin secretion in human subjects: daytime levels approach the limit of sensitivity of current radioimmunoassay methods; the nocturnal increase in serum melatonin levels begins near the onset of sleep and darkness at 11 pm; and peak values are reach around 4 hours later. When the individual melatonin patterns are viewed separately (Fig. 4), some very marked differences in peak height and total amounts of melatonin secreted are evident. Again, the small standard errors of the mean observed at corresponding time points when the melatonin profile is measured at different phases of the menstrual cycle, demonstrates remarkable replication of each subject's characteristic melatonin pattern despite the physiological variable.

DISCUSSION

These studies show that acute, day-long exposure of human subjects to contrasting lighting conditions that differ by as much as 25-fold in intensity, is without effect on the ensuing nocturnal increase in circulating melatonin levels; and that any relationship between the daily pattern of melatonin secretion and phases of the menstrual cycle is not discernible in a study of this kind. These studies further show (perhaps more significantly), that among normal healthy adult volunteers recruited from the general population and studied acutely in a clinical setting, there are distinct inter-individual differences in the phase, amplitude, and total amounts of melatonin rhythmically secreted; and that there is remarkable

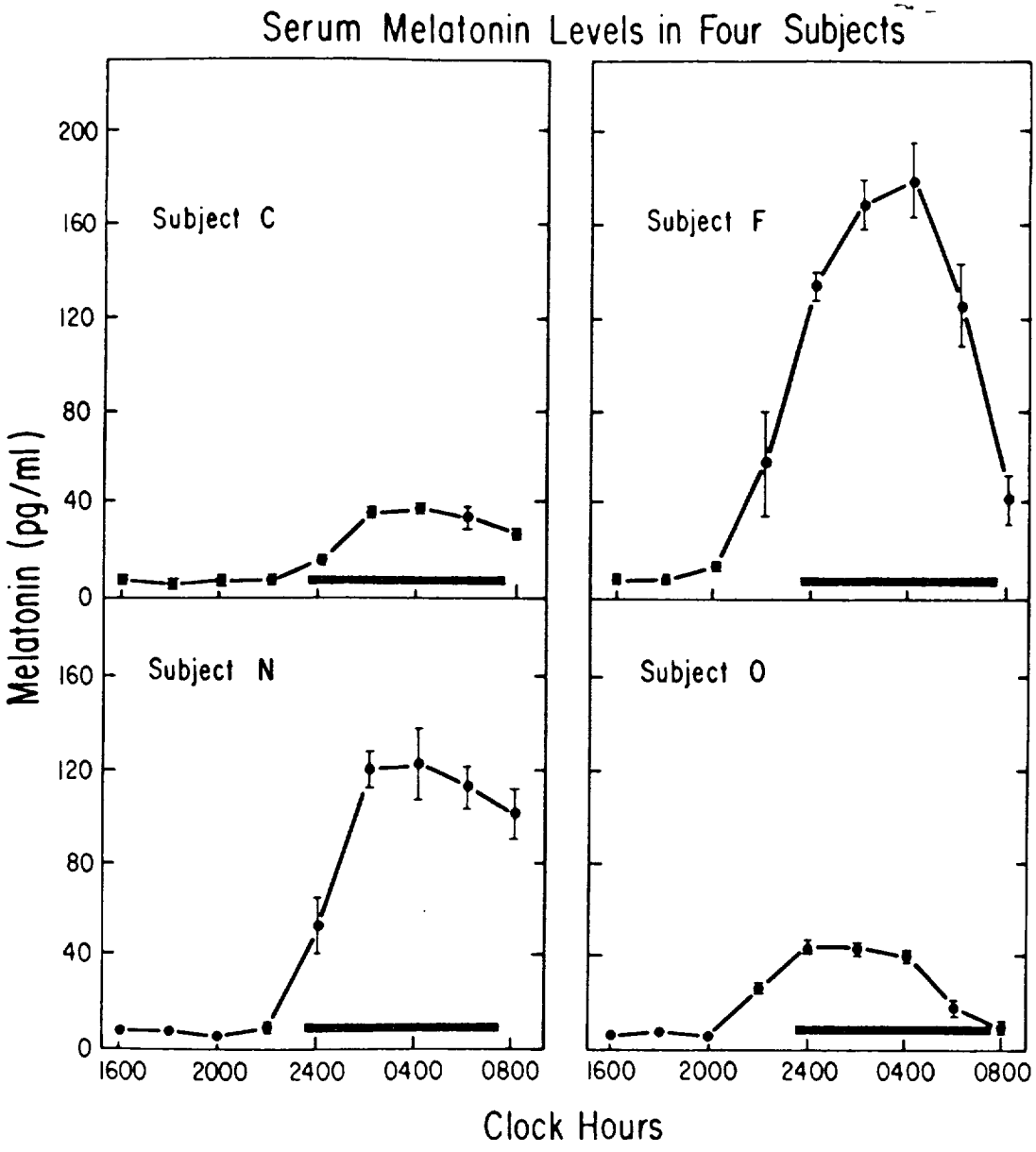

Fig. 2 Individual serum melatonin levels in four of the subjects represented in Figure 1, chosen to illustrate the diversity in that group. Each point represents the mean ± S.E. of melatonin levels measured at corresponding 2-hour intervals following daytime exposure to bright full spectrum light, dim full spectrum light or dim red light. Data from the three lighting conditions were pooled.

intra-individual consistency in these same parameters - despite experimental manipulation of the light intensity during the photoperiod or the physiological changes associated with the various phases of the menstrual cycle.

Since the very earliest studies of melatonin secretion in human subjects, such inter-individual variability and intra-individual consistency has been noted (Pelham, et al.,1973; Lynch, et al., 1975; Arendt, et al., 1975). In contrast, experimental studies of melatonin secretory patterns in laboratory animals (rats and hamsters) have yielded reasonably uniform and reproducible results. One possible reason for this difference between the results of human and animal studies may relate to the differences in experimental approaches to the study of melatonin rhythms in the two kinds of animals. Of necessity, groups of laboratory animals are killed at intervals to monitor temporal changes in melatonin levels.

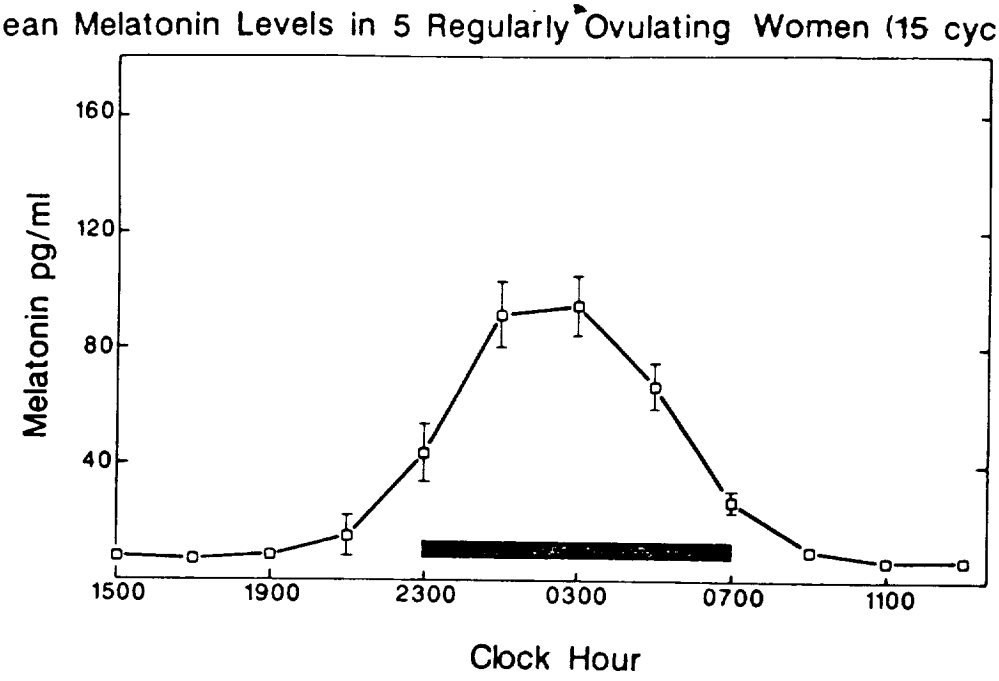

Fig. 3 Mean serum melatonin levels in 5 women sampled at 2-hour intervals during the follicular, periovulatory, and luteal phases of their menstrual cycles. Data from these three phases were pooled. Vertical bars represent one standard error of the mean. The horizontal bar indicates the period of sleep and darkness.

Individual rats or hamsters are not studied. Routinely, investigators obtain a homogeneous stock of animals and then acclimatize them, from one to three weeks, in a rigidly defined, timer controlled laboratory environment. Human subjects are typically recruited from the general population with due regard to sex, age and physical condition and then admitted to a standard clinical environment only very briefly for the purpose of collecting blood samples at intervals around the clock.

Most of our current understanding of the relationship between mammalian pineal function and environmental variables is based on studies involving rats and hamsters, nocturnal burrowing animals whose pineal function is exquisitely sensitive to very low light intensities and whose actual experience of environmental illumination we can only vaguely imagine. Recognizing the extreme contrast between the typical laboratory environment and the natural environment in which the wild ancestors of laboratory rats and hamsters evolved highly specialized adaptations (Hoffman, 1982; Lynch,et al., 1985), some investigators have studied the pattern of melatonin secretion among laboratory animals maintained in a naturalistic environment (e.g., using cages in which the animals had recourse to a dark burrow; Lynch, et al., 1982; Goldman, 1983). Such studies showed that when an animal has the opportunity to participate in determining its own light exposure, it experiences a very different photic environment from that typically imposed on animals in standard laboratory facilities. It is clear that either more exhaustive studies of animals in naturalistic environments, or field studies will be required to obtain a detailed understanding of how light influences rhythmic melatonin secretion in such animals. At the moment, field studies on pineal function in rats and hamsters are not technically feasible.

Curiously, the only mammal whose pineal function has been studied in the field is the human. Here we assert, that to transfer a person from his usual daily routine into a clinical research ward for a day or two, for the assessment of his melatonin secretory pattern, is essentially equivalent to studying him in the

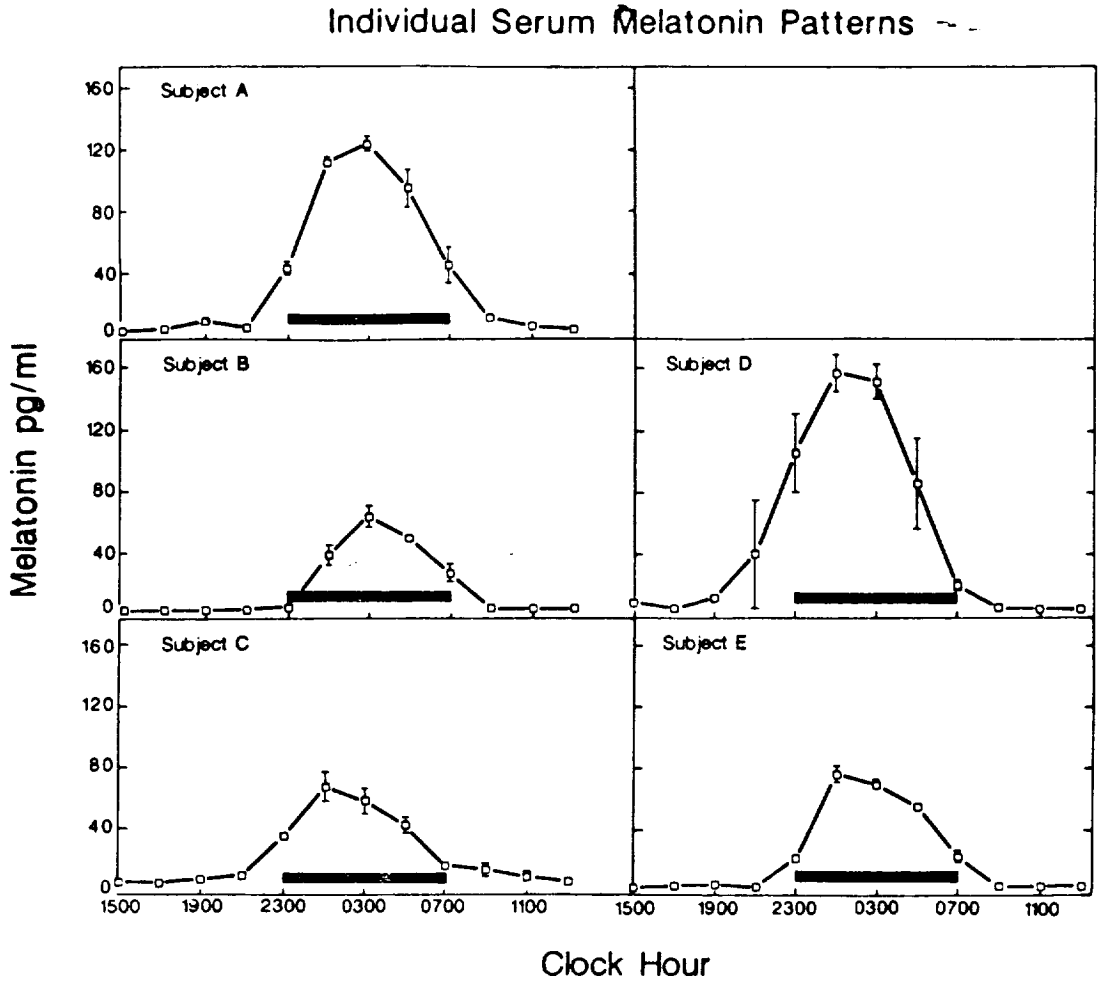

Fig. 4 Individual pooled serum melatonin levels for the 5 women represented in Figure 3. Each point represents the mean ± S.E. melatonin levels measured at corresponding 2-hour intervals on 3 says corresponding to a subject's follicular, periovulatory, and luteal menstrual phases.

field. The only analogous studies that have been done with animals are those recently reported by Reiter and his associates involving several species of diurnal rodents that were either captured in the wild or raised in the laboratory (Reiter and Peters, 1984; Reiter, et al., 1983; Reiter, et al., 1982). These studies showed, for example, vast differences between wild-captured and laboratory raised 13-lined ground squirrels in their rhythmic patterns of melatonin secretion and the susceptibility of these patterns to photic suppression (Reiter, et al., 1983). The animals were exposed either to ten hours of darkness at night or to light with an irradiance of 400 uW/cm2. In laboratory-raised squirrels, the period of darkness was associated with a gradual rise in melatonin levels that peaked 6 hours after the onset of darkness and then gradually declined to daytime levels. The exposure of laboratory-raised animals to an irradiance of 400 uW/cm2 during the night totally prevented the nocturnal rise in melatonin levels. In contrast, among wild-captured squirrels, the period of nocturnal darkness was associated with a rapid rise in melatonin levels which remained elevated throughout this period. Exposure of wild-captured animals to light (400 uW/cm2) during what would have been their normal dark period failed to suppress melatonin levels. These observations demonstrate that rhythmic melatonin secretion in this diurnal animal is a vastly mutable variable, and suggests that factors like the animal's previous photoperiodic experience may influence its nocturnal pattern of melatonin production.

These results are very similar to some of our own earlier observations on melatonin secretory patterns in humans. When volunteers were acutely challenged with sleep deprivation and overnight exposure to 500-900 lux of full spectrum illumination, their entrained rhythmic pattern of melatonin secretion remained unchanged. Also, in the same subjects, melatonin secretion was not provoked by an 8-hour period of sleep and darkness imposed in the middle of the normal daylight period following sleep deprivation and light exposure (Jimerson, et al., 1977). In men and chipmunks there is, thus, a degree of stability to the entrained rhythmic pattern of melatonin secretion which may be an expression of dynamic homeostasis. These entrained patterns are not immutable: When four volunteers admitted to a research ward, were first entrained to a rhythmic photic environment consisting of alternating 8-hour periods of darkness and 16-hour periods of light (900 lux), and then, abruptly phase shifted by 180o, each subject's plasma melatonin pattern did re-entrain to the new lighting regimen, over a period of 5 to 7 days (Lynch, et al., 1978). At that time, our analytical methods were not sufficiently sensitive to allow fine grained assessment of the time courses for their entrainment in each individual, nor to detect changes in the photic sensitivity of their melatonin patterns. Such work remains to be done.

We submit that volunteers for melatonin studies drawn from the general population and tested acutely are, in effect, "wild-captured" subjects. The diversity in amplitude, phase, and duration of their nightly melatonin secretion reflects the diversity in their individual life patterns. Some recent reports substantiate this view. In 1978 Vaughan reported that one subject who always slept in the afternoon and stayed awake all night had his plasma melatonin peak in the afternoon. Waldhauser described a similar case involving a student who habitually studied all night and slept during the day; when examined in a standard clinical setting, he displayed peak serum melatonin levels during the afternoon, in the light, and low melatonin levels, at night, in the dark, (Waldhauser and Dietzel, 1985). Waldhauser has since made additional and similar observations on two night-shift workers (personal communication). These, of course, are extreme cases. Might not more subtle environmental variables also be manifested in individual melatonin secretory patterns? By exposing a woman to four hours of bright light for seven consecutive evenings while her wake-sleep schedule remained unchanged, a 6-hour delay shift was induced in her daily patterns of body temperature and cortisol secretion (Czeisler, et al., 1986). By manipulating for several days their exposure to dim light before and after their usual nocturnal sleep period, the onset of night time melatonin production was effectively shifted in four subjects (Lewy, et al., 1985). What is the probability that the general population experiences such diversity in their routine habits and their exposure to light? In a study in which 10 healthy adult male and female subjects wore an apparatus on their foreheads which recorded their exposure to light as they went about their daily affairs, it was found that the subjects were exposed to daylight illumination intensities for only brief and scattered episodes during the 24-hour day. Some experienced brightest illumination in the morning and others in the evening (Okudaira, Kripke, and Webster, 1983).

It is perhaps surprising, under these circumstances, that it has been possible to develop any information relating human melatonin secretion to physiological and behavioral variables. Perhaps it would be useful to make a study of the genesis of human melatonin secretory patterns: Assess the homogeneity of melatonin patterns among distinctive groups, - such as shift workers, or people who characteristically experience either indoor or outdoor environments. It would also be useful to see if inter-individual variance in melatonin patterns might be diminished by acclimatizing normal volunteers to a behaviorally-constant, photically-defined, and temporally-structured, daily regimen. Such conditioned subjects might, like laboratory animals, constitute the appropriate population for

testing subtle effects on melatonin secretion of photic manipulations and other behavioral and physiological variables.

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